

## AMENDMENTS

### **In the claims:**

Please amend claims 1-3, 10-15, 19-22, 25, 27-28, 30-31, 34-35, 38, 42, and 47-48 as follows:

1. (Twice Amended) A method for obtaining a cell population enriched in antigen-specific T cells, comprising the steps of:

- a) exposing a cell population comprising T cells to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T cell and secretion of at least one product by the stimulated antigen-specific T cell, wherein the product is secreted in response to antigen stimulation of the antigen-specific T-cells;
- b) modifying the surface of the cells to contain a capture moiety specific for the product;
- c) culturing said population under conditions wherein said product is secreted, released and specifically bound to the capture moiety, thereby labeling the product-secreting cells; and
- d) separating the cells labeled with said secreted product to obtain a population of cells substantially enriched in antigen-specific T cells,

wherein steps a), b) and c) can be performed in any order or simultaneously.

2. (Once amended) A method according to claim 1, further comprising the step of labeling said secreted product specifically bound to the capture moiety prior to separation.

3. (Once amended) The method according to claim 2 wherein said secreted product is labeled with a label moiety.

10. (Once amended) The method according to claim 1 wherein said capture moiety is coupled to the cell surface of said T-cells through a lipid anchor attached to the capture moiety optionally through a linking moiety.

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11. (Once amended) The method according to claim 1 wherein said capture moiety is coupled to the cell surface of said T-cells through an antibody or an antigen-binding fragment thereof attached to the capture moiety, optionally through a linker.

12. (Once amended) The method according to claim 1 wherein said capture moiety is coupled to the cell surface of said T-cells through direct chemical coupling of the capture moiety to components on the cell surface, optionally through a linker.

13. (Once amended) The method according to claim 9 wherein said bispecific antibody is coupled to the cell surface of said T-cells through specific binding of the antibody to said cells.

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14. (Twice Amended) A method to label antigen-specific T cells with a product secreted and released by the cells, wherein the product is secreted in response to antigen stimulation of said T cells, which method comprises:

a) exposing the antigen-specific T cells to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T cell;

b) modifying the surface of the antigen-specific T cells to contain a capture moiety specific for the product; and

*D* c) culturing the cells <sup>wherein the cells are cultured</sup> under conditions wherein the product is secreted, released and specifically bound to the capture moiety, thereby labeling the product-secreting cells, <sup>and</sup> wherein steps a), b) and c) can be performed in any order or simultaneously.

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15. (Once amended) The method according to claim 14 wherein the secreted product specifically bound to the capture moiety is labeled with a label moiety.

6. 19. (Once amended) The method according to claim 14 wherein said capture moiety is coupled to the cell surface of said T-cells through a lipid anchor attached to the capture moiety optionally through a linker moiety.

7. 20. (Once amended) The method according to claim 14 wherein said capture moiety is coupled to the cell surface of said T-cells through an antibody or an antigen-binding fragment thereof attached to the capture moiety optionally through a linker. C6

8. 21. (Once amended) The method according to claim 18 wherein said bispecific antibody is coupled to the cell surface of said T-cells through specific binding of the antibody to the cell. 5

22. (Twice amended) A composition comprising cells labeled by the method according to claim 14, wherein said cells contain a capture moiety specific for a secreted product on their surface and a secreted product is specifically bound to said capture moiety.

25. (Once amended) The composition according to claim 22 wherein said capture moiety is coupled to the cell surface of said T-cells through a lipid anchor moiety attached to the capture moiety optionally through a linking moiety. C7

27. (Once amended) The composition according to claim 22 wherein said capture moiety is coupled to the cell surface of said T-cells through an antibody or an antigen-binding fragment thereof attached to the capture moiety, optionally through a linker. C8

28. (Once amended) The composition according to claim 24 wherein said antibody or an antigen-binding fragment thereof is coupled to the cell surface of said T-cells through specific binding of the antibody to the cell.

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30. (Once amended) Cells separated according to the method of claim 1, wherein said cells contain a capture moiety specific for a secreted product on their surface and a secreted product specifically bound to said capture moiety.

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31. (Twice Amended) A method of analyzing a population of cells to identify or enumerate antigen-specific T cells that secrete and release an amount of product relative to other cells in the population, wherein the product is secreted in response to antigen stimulation of the cells, the method comprising the steps of:

- a) labeling the cells by the method according to claim 14,
- b) labeling the cells with at least one additional label that does not label the captured product, and
- c) detecting the amount of product label relative to the additional label, wherein steps a), b) and c) can be performed in any order or simultaneously.

34. (Twice amended) A method for identifying antigen-specific T cells secreting and releasing at least one product in response to antigen stimulation of said cells, comprising the steps of:

- a) combining a mixed cell population comprising T cells with at least one first, bispecific, antibody, each antibody, having combining sites specific for a cell surface molecule of said T cells and at least one product;
- b) exposing the cell population to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T-cell;
- c) incubating the combination under conditions sufficient to allow the antigen-specific T-cell to secrete the at least one product, wherein said bispecific antibody specifically

binds to said cell surface molecule of said antigen-specific T-cell and specifically binds to at least one product secreted by said antigen-specific T-cell;

C11 d) adding at least one label moiety to said product secreted by said antigen-specific T-cell; and

e) detecting the at least one label moiety, wherein steps a), b), c), d) and e) can be performed in any order or simultaneously.

C12 35. (Once amended) The method according to claim 34 further comprising the step of separating the cells labeled with the product from the mixed cell population.

C13 38. (Once amended) The method according to claim 36 wherein the cell surface molecule is selected from the group consisting of CD2, CD3, CD4, CD5, CD8, CD11b, CD26, CD27, CD28, CD29, CD30, CD31, CD38, CD40L, CD45RO, CD45RA, LAG3, T1/ST2, SLAM, Class I MHC molecules, Class II MHC molecules, T cell antigen receptor, and  $\beta_2$ -microglobulin.

C14 42. (Once amended) The method according to claim 41 wherein the detectable label is selected from the group consisting of fluorophores, radioactive isotopes and chromophores.

C15 47. (Once amended) The method according to claim 46 wherein the detectable label is selected from the group consisting of fluorophores, radioactive isotopes and chromophores.

48. (Once amended) The method according to claim 47 wherein the detectable label is detected by fluorescence activated cell sorting.

Please add new claims 74-115 as follows:

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74. (New) A method for obtaining a cell population enriched in antigen-specific T cells, comprising the step of separating T cells labeled with a product secreted in response to antigen stimulation of the T-cells, from a cell population comprising T-cells, wherein said cell population comprising said T-cells has been exposed to at least one antigen specifically recognized by a T cell receptor and cultured under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T cell and secretion of at least one product by a stimulated antigen-specific T cell, wherein said product is secreted in response to antigen stimulation of the antigen-specific T-cells and wherein the surface of said cells has been modified to contain a capture moiety specific for said secreted product, and said secreted product specifically binds said capture moiety.

75. (New) A method according to claim 74, further comprising the step of labeling the secreted product specifically bound to the capture moiety prior to separation.

76. (New) The method according to claim 75 wherein the product is labeled with a label moiety.

77. (New) The method according to claim 76 wherein the label moiety is an antibody specific for the product.

78. (New) The method according to claim 76 wherein the label moiety is fluorochromated and the separation is conducted by cell sorting.

79. (New) The method according to claim 76 wherein the label moiety is magnetizable and the separation is conducted in a magnetic field of sufficient strength to magnetize the label moiety.

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80. (New) The method according to claim 79 wherein the label moiety comprises colloidal magnetic particles with a typical diameter of about 5 to 200 nm.

81. (New) The method according to claim 74 wherein the capture moiety is an antibody or an antigen-binding fragment thereof.

82. (New) The method according to claim 81 wherein the antibody or antigen binding fragment thereof is bispecific.

83. (New) The method according to claim 74 wherein said capture moiety is coupled to the cell surface of said T-cells through a lipid anchor attached to the capture moiety optionally through a linking moiety.

84. (New) The method according to claim 74 wherein said capture moiety is coupled to the cell surface of said T-cells through an antibody or an antigen-binding fragment thereof attached to the capture moiety, optionally through a linker.

85. (New) The method according to claim 74 wherein said capture moiety is coupled to the cell surface of said T-cells through direct chemical coupling of the capture moiety to components on the cell surface, optionally through a linker.

86. (New) The method according to claim 82 wherein said bispecific antibody or an antigen-binding fragment thereof is coupled to the cell surface of said T-cells through specific binding of the antibody to the cell.

87. (New) Cells obtained by the method of claim 74 wherein said cells contain a capture moiety on their surface and a product specifically bound to said capture moiety.

*10 88.* (New) A method to label antigen-specific T cells with a product secreted and released by the cells, wherein the product is secreted in response to antigen stimulation of said T

cells, which method comprises culturing antigen-specific T cells exposed to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen-specific T cell and secretion of said product by a stimulated antigen-specific T cell, wherein the surface of the antigen-specific T cell has been modified to contain a capture moiety, wherein said capture moiety is specific for the product and said product specifically binds said capture moiety, thereby labeling the product secreting cells.

~~11~~ <sup>10</sup>  
~~89.~~ (New) The method according to claim ~~88~~ wherein said antigen-specific T-cells exist within a population of cells.

~~12~~ <sup>10</sup>  
~~90.~~ (New) The method according to claim ~~88~~ wherein the secreted product is labeled with a label moiety.

~~13~~ <sup>12</sup>  
~~91.~~ (New) The method according to claim ~~90~~ wherein the label moiety is an antibody.

~~14~~ <sup>10</sup>  
~~92.~~ (New) The method according to claim ~~88~~ wherein the capture moiety is an antibody or an antigen-binding fragment thereof.

~~15~~ <sup>13</sup>  
~~93.~~ (New) The method according to claim ~~91~~ wherein the antibody is bispecific.

~~16~~ <sup>10</sup>  
~~94.~~ (New) The method according to claim ~~88~~ wherein said capture moiety is coupled to the cell surface of said T-cells through a lipid anchor attached to the capture moiety optionally through a linker moiety.

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95. (New) The method according to claim ~~88~~ wherein said capture moiety is coupled to the cell surface of said T-cells through an antibody or an antigen-binding fragment thereof attached to the capture moiety optionally through a linker.

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96. (New) The method according to claim ~~93~~ wherein said bispecific antibody is coupled to the cell surface of said T-cells through specific binding of the antibody to the cell.

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97. (New) The method according to claim ~~14~~, wherein said antigen-specific T-cells exist within a population of cells.

*C10*

98. (New) Antigen-specific T cells labeled by the method of claim 88 wherein said cells contain a capture moiety and a product specifically bound to said capture moiety.

99. (New) A method for identifying antigen-specific T cells secreting and releasing at least one product in response to antigen stimulation of said cells, comprising the steps of detecting a label moiety contained on a product secreted by a T cell in a mixed cell population, wherein said cell population has been combined with at least one first, bispecific, antibody, each antibody, having combining sites specific for a cell surface molecule of said T-cells and at least one product, wherein said population of cells has been exposed to at least one antigen specifically recognized by a T cell receptor under conditions effective to elicit antigen-specific stimulation of at least one antigen specific T cell and secretion of said product by said antigen-specific T cell, wherein said bispecific antibody specifically binds to said cell surface molecule of said antigen-specific T-cell and specifically binds to at least one product secreted by said antigen-specific T-cells and wherein said product has been labeled with a label moiety.

100. The method according to claim 99 further comprising the step of separating the cells secreting the product from the mixed cell population.

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101. The method according to claim 99 wherein the cell surface molecule is a naturally occurring cell surface protein.

102. The method according to claim 101 wherein the protein is a cell surface marker.

103. The method according to claim 99 wherein the cell surface molecule is selected from the group consisting of CD2, CD3, CD4, CD5, CD8, CD11b, CD26, CD27, CD28, CD29, CD30, CD31, CD38, CD40L, CD45RO, CD45RA, LAG3, T1/ST2, SLAM, Class I MHC molecules, Class II MHC molecules, T cell antigen receptor, and  $\beta_2$ -microglobulin.

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104. The method according to claim 99 wherein the incubation conditions include a high viscosity or gel forming medium.

105. The method according to claim 99 wherein the label moiety is an antibody.

106. The method according to claim 105 wherein the antibody comprises a detectable label.

107. The method according to claim 106 wherein the detectable label is selected from the group consisting of fluorophores, radioactive isotopes and chromophores.

108. The method according to claim 105 wherein the label moiety is detected by fluorescence activated cell sorting.

109. The method according to claim 108 wherein the label moiety is detected by a third antibody.

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110. The method according to claim 109 wherein the label moiety is coupled to digoxigenin and the third antibody is specific for digoxigenin.

111. The method according to claim 110 wherein the third antibody comprises a detectable label.

112. The method according to claim 111 wherein the detectable label is selected from the group consisting of fluorophores, radioactive isotopes and chromophores.

113. The method according to claim 112 wherein the label moiety is detected by fluorescence activated cell sorting.

114. The method according to claim 99 wherein the label moiety comprises a magnetizable moiety.

115. The method according to claim 114 wherein the label moiety is detected by a third antibody coupled to a magnetizable moiety.